

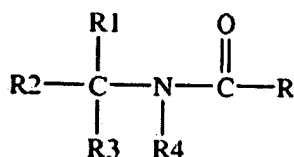
10/529115  
JC17 Rec'd PCT/PTO 24 MAR 2005

**To the claims:**

**Please amend Claims 8, 29 and 59.**

**The currently pending and amended claims are below. Please amend the claims following, wherein the deleted matter is shown by strikethrough and the added matter is shown by underlining.**

1. (Previously presented) A method of producing a human neural cell comprising,
  - a) providing a pluripotent human cell; and
  - b) culturing the pluripotent human cell with a composition comprising a ceramide compound of the general formula



wherein

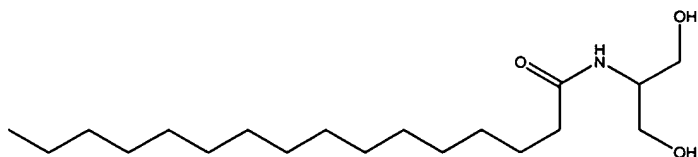
R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen

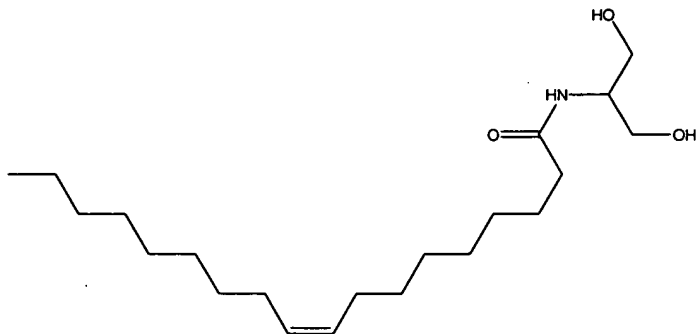
to produce the human neural cell.

2. (Previously presented) The method of Claim 1, wherein the pluripotent human cell is a differentiating pluripotent human cell.
3. (Previously presented) The method of Claim 1, comprising the intermediate step of forming an embryoid body comprising the pluripotent human cell prior to culturing a cell from the embryoid body with the ceramide compound.
4. (Previously presented) The method of Claim 3, wherein the embryoid body is formed by culturing the pluripotent human cell with an essentially serum free medium.

5. (Previously presented) The method of Claim 4, wherein the essentially serum free medium is a MEDII conditioned medium.
6. (Previously presented) The method of Claim 5, comprising the additional steps of,
  - a) dispersing the embryoid body to an essentially single cell suspension;
  - b) culturing the essentially single cell suspension comprising the pluripotent human cell in an adherent culture with a composition comprising the ceramide compound.
7. (Previously presented) The method of Claim 6, wherein the composition comprising the ceramide compound further comprises a MEDII conditioned medium.
8. (Currently amended) The method of ~~any of~~ Claim[s] 5 [~~6, or 7,~~] wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
9. (Previously presented) The method of Claim 7, wherein the composition comprising the ceramide compound is essentially serum free.
10. (Previously presented) The method of Claim 1, wherein the composition comprises a ceramide compound of the structure



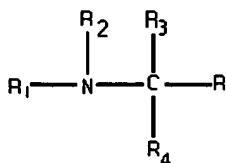
11. (Previously presented) The method of Claim 1, wherein the composition comprises a ceramide compound of the structure



12. (Previously presented) The method of Claim 1, wherein the concentration of the ceramide compound is from approximately 0.1  $\mu\text{M}$  to approximately 1000  $\mu\text{M}$ .

13. (Previously presented) The method of Claim 1, wherein the concentration of the ceramide compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .
14. (Previously presented) The method of Claim 1, wherein the concentration of the ceramide compound is from approximately 5  $\mu\text{M}$  to approximately 50  $\mu\text{M}$ .
15. (Previously presented) The method of Claim 1, wherein the concentration of the ceramide compound is approximately 10  $\mu\text{M}$ .
16. (Previously presented) The method of Claim 1, wherein the duration of culturing the human pluripotent cell with the ceramide compound is from approximately 6 hours to 10 days.
17. (Previously presented) The method of Claim 1, wherein R= a saturated or mono- or polyunsaturated (cis or trans) alkyl group having from 12-20 carbon atoms.
18. (Previously presented) The method of Claim 17, wherein the hydroxylated alkyl groups have from 1-6 carbon atoms.
19. (Previously presented) The method of Claim 18, wherein R1 and R2 are hydroxylated alkyl groups.
20. (Previously presented) The method of Claim 1, wherein the pluripotent human cell is selected from the group consisting of a human embryonic stem cell, a human inner cell mass (ICM)/epiblast cell, a human primitive ectoderm cell, and a human primordial germ cell.
21. (Previously presented) The method of Claim 1, wherein the pluripotent human cell is a human embryonic stem cell.
22. (Previously presented) The method of Claim 1, wherein the human pluripotent cell is a multipotent cell.
23. (Previously presented) The method of Claim 22, wherein the multipotent cell is a neural precursor cell.
24. (Previously presented) A method of producing a human neural cell comprising,
  - a) providing a pluripotent human cell;

- b) forming an embryoid body comprising the pluripotent human cell by culturing the pluripotent human cell in a medium that is optionally essentially serum free; and
- c) culturing cells from the embryoid body with a composition comprising a sphingosine compound of the general formula



wherein

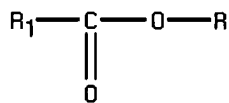
R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen.

25. (Previously presented) The method of Claim 24, wherein the medium is an essentially serum free medium and comprises a MEDII conditioned medium.

26. (Previously presented) A method of producing a human neural cell comprising,

- a) providing a pluripotent human cell;
- b) forming an embryoid body comprising the pluripotent human cell by culturing the pluripotent human cell in a medium that is optionally essentially serum free; and
- c) culturing cells from the embryoid body with a composition comprising a hydroxyalkyl ester compound of the general formula



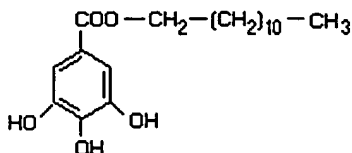
wherein

R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1 is a saturated or mono-or polyunsaturated hydroxylated alkyl group, aryl group, or hydrogen.

27. (Previously presented) The method of Claim 26, wherein the medium is an essentially serum free medium and comprises a MEDII conditioned medium.

28. (Previously presented) The method of Claim 27, wherein the composition comprises an hydroxyalkyl ester compound of the structure



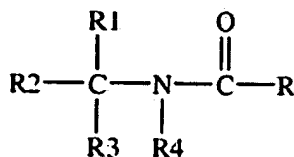
29. (Currently amended) A neural cell produced by ~~any of~~ the method[s] of Claim[s] 1[-28].

30. (Previously presented) A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the neural cell of Claim 29.

31. (Previously presented) The method of Claim 30, wherein the neural disease is Parkinson's disease.

32. (Previously presented) A method of enhancing the efficiency of the transplantation of a cultured human pluripotent cell in a patient, comprising the steps of:

- a) culturing a human pluripotent cell with a growth medium comprising a ceramide compound of the formula



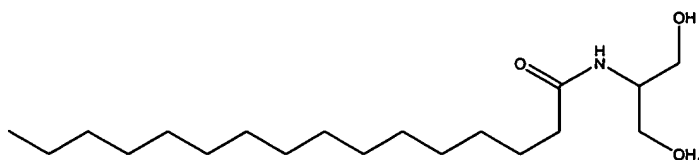
wherein

R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

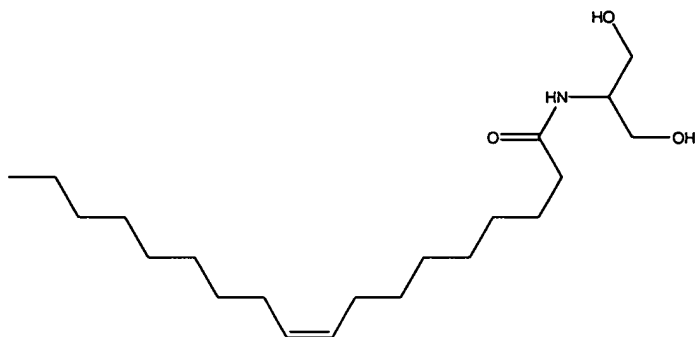
R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen; and

- b) transplanting the cultured human pluripotent cell into the patient.

33. The method of Claim 32, wherein the composition comprises a ceramide compound of the structure



34. (Previously presented) The method of Claim 32, wherein the composition comprises a ceramide compound of the structure



35. (Previously presented) The method of Claim 32, wherein the duration of step (a) is for a period of from approximately 6 hours to 10 days.

36. (Previously presented) The method of Claim 35, wherein the cell population comprising the cultured human pluripotent cell contains at least 80% of a neural cell.

37. (Previously presented) The method of Claim 32, wherein the concentration of the ceramide compound is from approximately 0.1  $\mu\text{M}$  to approximately 1000  $\mu\text{M}$ .

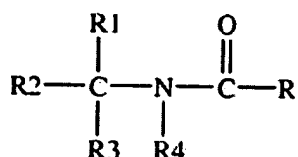
38. (Previously presented) The method of Claim 32, wherein the concentration of the ceramide compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .

39. (Previously presented) The method of Claim 32, wherein the concentration of the ceramide compound is from approximately 5  $\mu$ M to approximately 50  $\mu$ M.

40. (Previously presented) The method of Claim 32, wherein the concentration of the ceramide compound is approximately 10  $\mu$ M.

41. (Previously presented) The method of Claim 32, wherein R= a saturated or mono- or polyunsaturated (cis or trans) alkyl group having from 12-20 carbon atoms.

42. (Previously presented) The method of Claim 41, wherein the hydroxylated alkyl groups have from 1-6 carbon atoms.
43. (Previously presented) The method of Claim 42, wherein R1 and R2 are hydroxylated alkyl groups.
44. (Previously presented) A composition for promoting maintenance, proliferation, or differentiation of a human neural cell, the composition comprising a cell culture medium comprising MEDII conditioned medium and a ceramide compound of the general formula

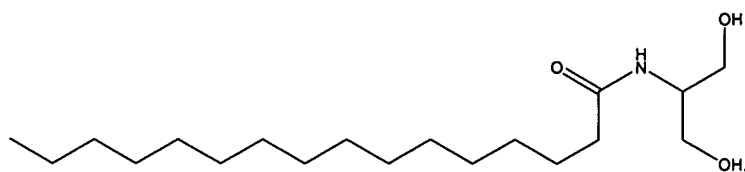


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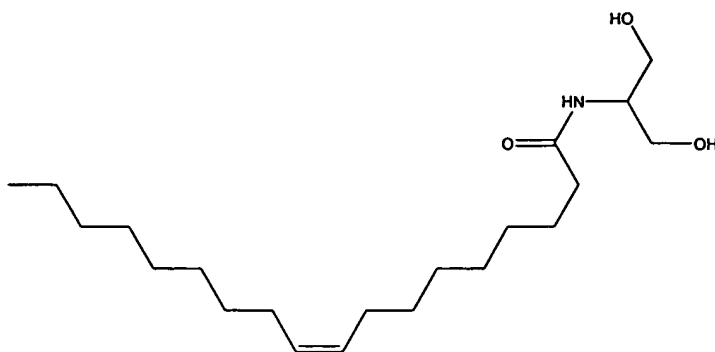
R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen.

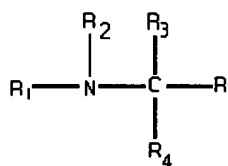
45. (Previously presented) The composition of Claim 44, wherein the composition comprises a ceramide compound of the formula



46. (Previously presented) The composition of Claim 44, wherein the composition comprises a ceramide compound of the formula



47. (Previously presented) The composition of Claim 44, wherein the concentration of the ceramide compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .
48. (Previously presented) The composition of Claim 44, wherein the concentration of the ceramide compound is approximately 10  $\mu\text{M}$ .
49. (Previously presented) The composition of Claim 44, wherein the human neural cell is cultured with the composition for a period of from approximately 6 hours to 10 days.
50. (Previously presented) A composition for promoting maintenance, proliferation, or differentiation of a human neural cell, the composition comprising a cell culture medium comprising MEDII conditioned medium and a sphingosine compound of the general formula



wherein

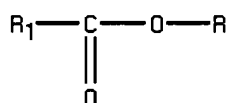
R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and  
R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen.

51. (Previously presented) The composition of Claim 50, wherein the concentration of the sphingosine compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .
52. (Previously presented) The composition of Claim 50, wherein the concentration of the sphingosine compound is approximately 10  $\mu\text{M}$ .



53. (Previously presented) The composition of Claim 50, wherein the human neural cell is cultured with the composition for a period of from approximately 6 hours to 10 days.

54. (Previously presented) A composition for promoting maintenance, proliferation, or differentiation of a human neural cell, the composition comprising a cell culture medium comprising MEDII conditioned medium and a hydroxyalkyl ester compound of the general formula

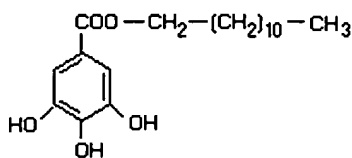


wherein

R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1 is a saturated or mono-or polyunsaturated hydroxylated alkyl group, aryl group, or hydrogen.

55. (Previously presented) The composition of Claim 54, wherein the composition comprises a hydroxyalkyl ester compound of the formula



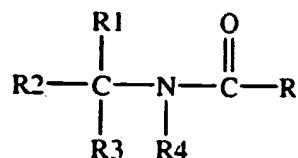
56. (Previously presented) The composition of Claim 55, wherein the concentration of the hydroxyalkyl ester compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .

57. (Previously presented) The composition of Claim 55, wherein the concentration of the hydroxyalkyl ester compound is approximately 10  $\mu\text{M}$ .

58. (Previously presented) The composition of Claim 55, wherein the human neural cell is cultured with the composition for a period of from approximately 6 hours to 10 days.

59. (Currently amended) A neural cell cultured in the composition of ~~any of~~ Claim[s] 44[-58].

60. (Previously presented) A cell culture composition comprising a differentiating pluripotent human cell and a composition comprising a ceramide compound of the general formula

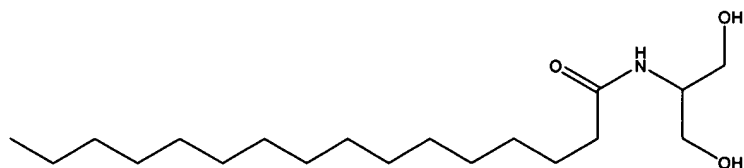


wherein

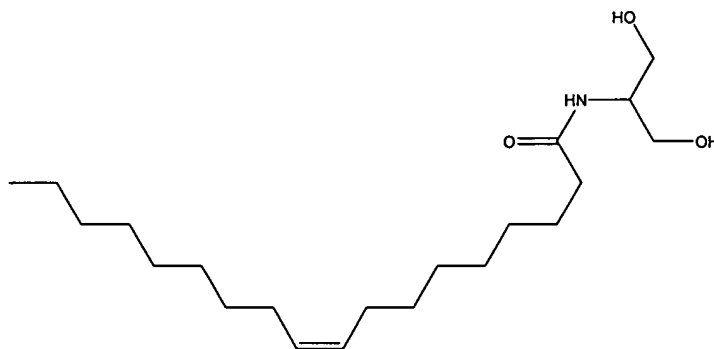
R=a saturated or mono- or polyunsaturated (cis or trans) alkyl group having greater than 2 carbon atoms, and

R1, R2, R3 and R4 may be the same or different and are saturated or mono-or polyunsaturated hydroxylated alkyl groups, aryl groups, or hydrogen.

61. (Previously presented) The composition of Claim 60, wherein the composition comprises a ceramide compound of the formula



62. (Previously presented) The composition of Claim 60, wherein the composition comprises a ceramide compound of the formula



63. (Previously presented) The composition of Claim 60, wherein the concentration of the ceramide compound is from approximately 1  $\mu\text{M}$  to approximately 100  $\mu\text{M}$ .

64. (Previously presented) The composition of Claim 60, wherein the concentration of the ceramide compound is approximately 10  $\mu$ M.
65. (Previously presented) The composition of Claim 60, wherein the differentiating pluripotent human cell is cultured with the composition for a period of from approximately 6 hours to 10 days.
66. (Previously presented) The method of Claim 60, wherein R= a saturated or mono- or polyunsaturated (cis or trans) alkyl group having from 12-20 carbon atoms.
67. (Previously presented) The method of Claim 66, wherein the hydroxylated alkyl groups have from 1-6 carbon atoms.
68. (Previously presented) The method of Claim 67, wherein R1 and R2 are hydroxylated alkyl groups.
69. (Previously presented) The method of Claim 60, wherein the pluripotent human cell is selected from the group consisting of a human embryonic stem cell, a human inner cell mass (ICM)/epiblast cell, a human primitive ectoderm cell, and a human primordial germ cell.
70. (Previously presented) The method of Claim 60, wherein the pluripotent human cell is a human embryonic stem cell.
71. (Previously presented) The method of Claim 60, wherein the differentiating human pluripotent cell is a neural precursor cell.